

Involvement of PAF in postallergic propranolol-induced bronchoconstriction in guinea-pigs

M. Fujimura, M. Tsujiura, N. Songür, S. Myou, T. Matsuda

Involvement of PAF in postallergic propranolol-induced bronchoconstriction in guinea-pigs. M. Fujimura, M. Tsujiura, N. Songür, S. Myou, T. Matsuda. ©ERS Journals Ltd 1996.

ABSTRACT: Administration of propranolol can provoke bronchoconstriction in asthmatic patients. Recently, we successfully developed a guinea-pig model for propranolol-induced bronchoconstriction (PIB). We hypothesized that such bronchoconstriction may result from the inflammatory mediators released by an allergic reaction. The purpose of this study was to examine the role of platelet-activating factor (PAF) in the development of PIB after allergic reaction.

Propranolol, at a concentration of 10 mg·mL⁻¹ was inhaled 20 min after antigen challenge in passively sensitized, anaesthetized and artificially-ventilated guinea-pigs. The animals were treated intravenously with PAF antagonists, E6123 (1 and 10 µg·kg⁻¹) or Y-24180 (1 and 10 mg·kg⁻¹), 10 min before or 15 min after antigen challenge.

Propranolol inhaled 20 min after antigen challenge caused bronchoconstriction. E6123 and Y-24180 administered 15 min after antigen challenge as well as 10 min before antigen challenge reduced the PIB in a dose-dependent manner.

We conclude that platelet-activating factor may contribute to the development of propranolol-induced bronchoconstriction after allergic reaction in our guinea-pig model.

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Propranolol can cause bronchoconstriction in asthmatic patients, whether it is administered orally, intravenously or by inhalation [1, 2]. Propranolol challenge of asthmatic patients leads to bronchoconstriction, usually within 10 min [3, 4]. The reaction can be severe, prolonged, and difficult to reverse. We recently developed an animal model in which propranolol provokes bronchoconstriction within a few minutes when propranolol was inhaled 20 min after challenge with aerosolized antigen in passively sensitized and artificially ventilated guinea-pigs [5]. Propranolol inhalation did not cause bronchoconstriction 5 or 20 min after methacholine-induced bronchoconstriction in nonsensitized guinea-pigs, or 20 min after saline inhalation in passively sensitized animals [5]. In addition, a dual neurokinin-1 (NK₁) and neurokinin-2 (NK₂) antagonist, FK224, attenuated nonspecific bronchial hyperresponsiveness to methacholine induced by allergic reaction [6], but did not change the propranolol-induced bronchoconstriction (PIB) (unpublished data). Consequently, we consider that allergic reaction but not bronchoconstriction specifically leads to PIB.

The pathogenesis of PIB is unclear. Some researchers have proposed that cholinergic nerve activity may play a role [7]. Mast cells in human lung possess β-receptors on their surface [8]. Beta-adrenoceptor agonists have been shown to inhibit the anaphylactic release of mediators, including histamine, slow-reacting substance of anaphylaxis (SRS-A; leukotriene (LT) C₄, D₄, and E₄) and thromboxane A₂ (TxA₂) [9]. Histamine was found

to be involved in the PIB in asthmatic patients [10]. Our previous studies [5, 11] showed that thromboxane A₂ and 5-lipoxygenase products are important in the PIB. These findings suggest that inflammatory mediators contribute to the development of PIB.

PAF is a highly potent inflammatory mediator with a wide range of activities. PAF is produced by many different cells. A particular property of PAF that may be relevant to asthma is its capacity to induce eosinophilic inflammation [12]. It has potent effects on the microvasculature and may also contribute to bronchial hyperresponsiveness by an effect on airway β-adrenoceptors [13].

The purpose of this study was to examine whether PAF is involved in the development of PIB. The effect of two different PAF antagonists, E6123 [14] and Y-24180 [15], on the PIB following aerosolized antigen-induced bronchoconstriction in guinea-pigs was assessed.

Materials and methods

Animals

Male albino, Hartley strain guinea-pigs, weighing 350–400 g, were obtained from Sankyō Laboratory Service (Toyama, Japan). After arrival at the Institute of Animal

Experiments in our university, they were kept in conventional animal housing facilities for 1 week before use. They were allowed to drink and feed *ad libitum*.

Passive sensitization of guinea-pigs

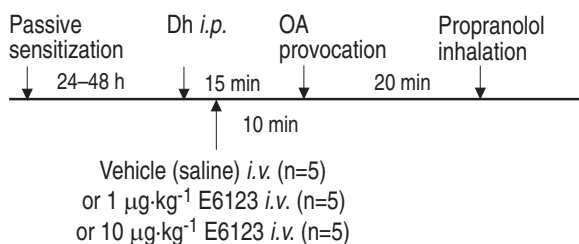
Guinea-pig homocytotropic antiserum was obtained by the method of SANTIVES *et al.* [16]. Briefly, 500 μg of ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally into each guinea-pig at multiple sites. A booster dose was prepared and administered in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster dose, pooled, and kept frozen until use. The antibody titre of this serum was 1:12800, 1:6400, and 1:512, as estimated by passive cutaneous anaphylaxis at 4 and 24 h, and 7 days, respectively. Normal guinea-pigs were passively sensitized with 1.0 mL antiserum·kg⁻¹ intraperitoneally.

Preparation of animals

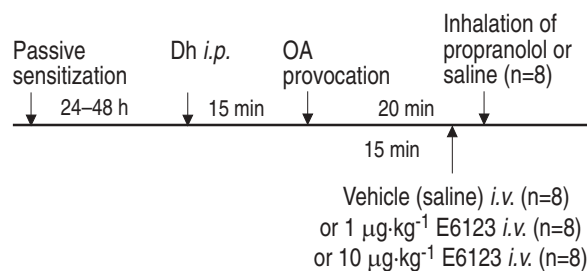
The experimental study design is presented in figure 1. Between 24 and 48 h after the passive sensitization, guinea-pigs were anaesthetized with sodium penobarbital (75 mg·kg⁻¹ *i.p.*). They were placed in the supine position, and the trachea was cannulated with a polyethylene tube (external diameter 2.5 mm, internal diameter 2.1 mm). The left jugular vein was cannulated for administration of drugs.

Study 1. Effect of E6123 on the propranolol-induced bronchoconstriction

E6123 treatment before antigen challenge



E6123 treatment after antigen challenge



After surgery, each guinea-pig was artificially ventilated by a small animal respiratory pump (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA, USA). The tidal volume was 10 mL·kg⁻¹ and the rate was 60 strokes·min⁻¹. The changes in lung resistance to inflation, defined as the lateral pressure of the tracheal tube or pressure at the airway opening (P_{ao}) was measured using a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co. Ltd., Tokyo, Japan). The modified method of KONZETT and ROSSLER [17], described by JONES *et al.* [18], was used to measure these changes. Since we [18] demonstrated that the change in P_{ao} following inhalation of LTC₄ represented the average of the changes in pulmonary resistance and reciprocal dynamic lung compliance, P_{ao} was used as an overall index of the bronchial response to bronchoactive agents.

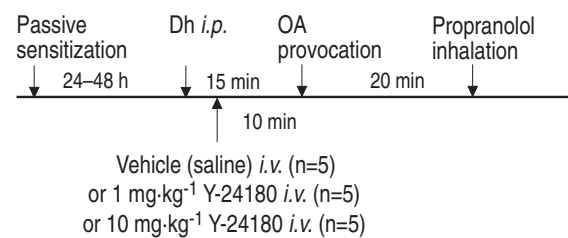
When all procedures were completed, the animals were administered diphenhydramine hydrochloride (60 mg·kg⁻¹ *i.p.*) to block the action of histamine. By clamping the outlet port of the respirator, the animals were overinflated by two times tidal volume for two breaths [19]

Propranolol-induced bronchoconstriction

Fifteen minutes after the preparation, when P_{ao} had been stabilized, the animals were challenged with nebulized ovalbumin (OA) dissolved in saline (1.0 mg·mL⁻¹) without interrupting the constant ventilation in passively sensitized animals. The OA aerosol was generated for 30 s with an ultrasonic nebulizer developed for small animals at our institution [20]. The amount of aerosol

Study 2. Effect of Y-24180 on the propranolol-induced bronchoconstriction

Y-24180 treatment before antigen challenge



EY-24180 treatment after antigen challenge

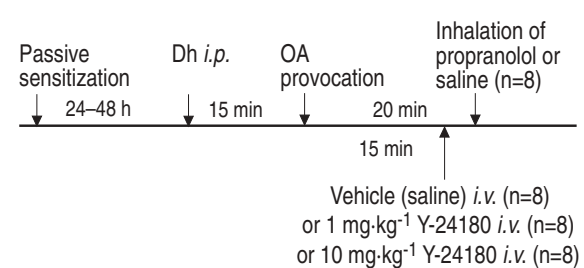


Fig. 1. — Experimental study design. Dh: diphenhydramine hydrochloride; OA: ovalbumin.

was $15.2 \mu\text{L}\cdot\text{min}^{-1}$, and 46.4% of the aerosol was deposited in the lung as measured by radioaerosol technique [20]. Twenty minutes after the OA provocation, $10 \text{ mg}\cdot\text{mL}^{-1}$ of propranolol was inhaled for 30 s.

Study 1: Effect of E6123 on propranolol-induced bronchoconstriction

E6123 treatment before antigen challenge. A PAF antagonist E6123 at a dose of 1 (n=5) or $10 \mu\text{g}\cdot\text{kg}^{-1}$ (n=5) dissolved in saline, or the vehicle (saline) (n=5), was administered *i.v.* 10 min before the OA challenge; 30 min before propranolol inhalation.

E6123 treatment after antigen challenge. E6123 at a dose of 1 (n=8) or $10 \mu\text{g}\cdot\text{kg}^{-1}$ (n=8), or vehicle (saline) (n=8), was given *i.v.* 15 min after the challenge with OA and propranolol was inhaled 5 min later; 20 min after the OA challenge. As a negative control, saline was given *i.v.* 15 min after the challenge with OA and saline was inhaled 5 min later (n=8).

Study 2: Effect of Y-24180 on propranolol-induced bronchoconstriction

Y-24180 treatment before antigen challenge. A PAF antagonist Y24180 at a dose of 1 (n=5) or $10 \text{ mg}\cdot\text{kg}^{-1}$ (n=5) dissolved in saline, or the vehicle (saline) (n=5), was administered *i.v.* 10 min before the OA challenge; 30 min before propranolol inhalation.

Y-24180 treatment after antigen challenge. Y-24180 at a dose of 1 (n=8) or $10 \text{ mg}\cdot\text{kg}^{-1}$ (n=8), or vehicle (saline), (n=8) was given *i.v.* 15 min after the challenge with OA and then propranolol inhalation was performed 5 min later; 20 min after the OA challenge. As a negative control, saline was given *i.v.* 15 min after the challenge with OA and saline was inhaled 5 min later (n=8).

Statistical analysis

All data are shown as mean \pm SEM. Statistical differences were determined by nonparametric analysis of variance (ANOVA) among three groups and Mann Whitney's U-test between two groups. Differences of time course curves for percentage increase in P_{ao} from the baseline value after OA provocation and inhalation of propranolol were analyzed among animals treated with E6123 (1 and $10 \mu\text{g}\cdot\text{kg}^{-1}$) and vehicle or Y-24180 (1 and $10 \text{ mg}\cdot\text{kg}^{-1}$) and vehicle with 2-factor repeated ANOVA. A p-value of 0.05 or less was considered to be significant.

Chemicals

The following chemicals were used: ovalbumin (Sigma, St Louis, USA), diphenhydramine hydrochloride (Sigma, St Louis, USA), sodium pentobarbital (Abbot Laboratories, North Chicago, USA), dl-propranolol hydrochloride (Wako Pure Chemical Industries Ltd, Osaka, Japan),

dimethyl sulphoxide (DMSO) (Wako Pure Chemical Industries Ltd, Osaka, Japan), E6123 ((S)-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8H-pyrido[4,3':4,5]thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine) (Eisai Co. Ltd., Tokyo, Japan), and Y-24180 (4-(2-chlorophenyl)-2-(2-(4-isobutylphenyl)ethyl)-6,9-dimethyl-6H-thieno-(3,2-f)(1,2,4)triazolo-(4,3-a)(1,4) diazepine) (Yoshitomi Pharmaceutical Industries Ltd., Osaka, Japan).

Results

Study 1: Effect of E6123 on propranolol-induced bronchoconstriction

E6123 treatment before antigen challenge. P_{ao} values before ovalbumin (OA) challenge were 10.1 ± 0.1 , 10.2 ± 0.1 and $10.2 \pm 0.2 \text{ cmH}_2\text{O}$ with pretreatment with 1 and $10 \mu\text{g}\cdot\text{kg}^{-1}$ of E6123 and vehicle (saline), respectively. There were no significant differences between them. The time courses of percentage increase in P_{ao} from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in figure 2. The curves both for antigen-induced bronchoconstriction (0–20 min after OA challenge) and propranolol-induced bronchoconstriction (20–35 min after OA challenge) were significantly inhibited by E6123 in a dose-dependent manner ($p < 0.01$ and $p < 0.05$ between three groups by two-factor repeated ANOVA). The peak values from continuous measurement after OA challenge were 256 ± 4 , 170 ± 23 and $120 \pm 26\%$ with vehicle and 1 and $10 \mu\text{g}\cdot\text{kg}^{-1}$ of E6123, respectively, and the value was significantly lower with 1 ($p < 0.01$) and $10 \mu\text{g}\cdot\text{kg}^{-1}$ of E6123 ($p < 0.01$) than with vehicle. The maximum percentage increase in P_{ao} from continuous measurement after propranolol inhalation from the pre-OA challenge value were 392 ± 14 , 294 ± 36 and $242 \pm 41\%$ with vehicle and

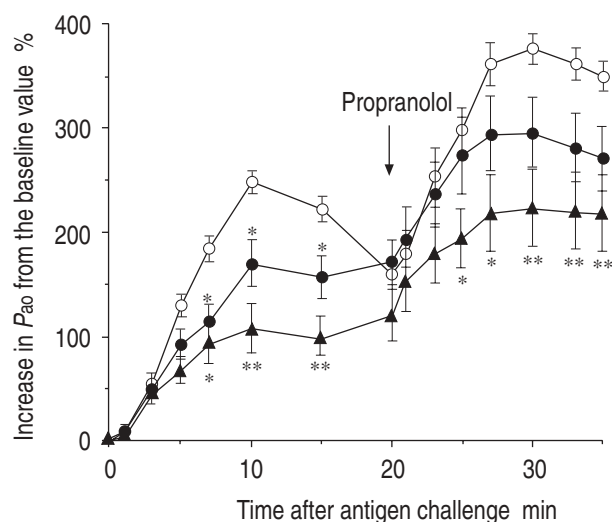


Fig. 2. — Time course of percentage increase in pressure at the airway opening (P_{ao}) in passively sensitized guinea-pigs pretreated *i.v.* with vehicle (saline) (n=5), $1 \mu\text{g}\cdot\text{kg}^{-1}$ E6123 (n=5), or $10 \mu\text{g}\cdot\text{kg}^{-1}$ E6123 (n=5) 10 min before ovalbumin challenge. Vertical bars represent SEM. *: $p < 0.05$; **: $p < 0.01$, compared with the vehicle treatment group. —○—: vehicle (n=5); —●—: E6123 $1 \mu\text{g}\cdot\text{kg}^{-1}$ (n=5); —▲—: E6123 $10 \mu\text{g}\cdot\text{kg}^{-1}$ (n=5).

1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123, respectively. The value with 1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123 was significantly ($p<0.05$ and $p<0.01$, respectively) lower than that with vehicle. As shown in figure 2, 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123 significantly inhibited the increases in P_{ao} both after OA challenge and after propranolol inhalation.

E6123 treatment after antigen challenge P_{ao} values before OA provocation were 10.2 ± 0.2 , 10.1 ± 0.1 and 10.0 ± 0.1 cmH_2O in animals pretreated with 1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123 and vehicle, respectively. There were no significant differences between them. The time courses of percentage increase in P_{ao} from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in figure 3. The peak values from continuous measurement after OA challenge were 354 ± 27 , 288 ± 53 and $328\pm 22\%$ with vehicle and 1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123, respectively, and there were no significant differences between them. Percentage increase in P_{ao} immediately before propranolol inhalation (20 min after the OA challenge), determined from continuous measurement were 261 ± 29 , 240 ± 64 and $265\pm 42\%$ in the groups of vehicle, 1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123, respectively. These values were not significantly different. Although the time courses of percentage increase in P_{ao} after the inhalation of propranolol were not significantly different between the three groups, the maximum percentage increase in P_{ao} from continuous measurement after propranolol inhalation from the pre-OA challenge value were 481 ± 24 , 413 ± 39 and $355\pm 33\%$ with vehicle and 1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123, respectively. The value with 10 $\mu\text{g}\cdot\text{kg}^{-1}$ of E6123 was significantly ($p<0.05$) lower than that with vehicle.

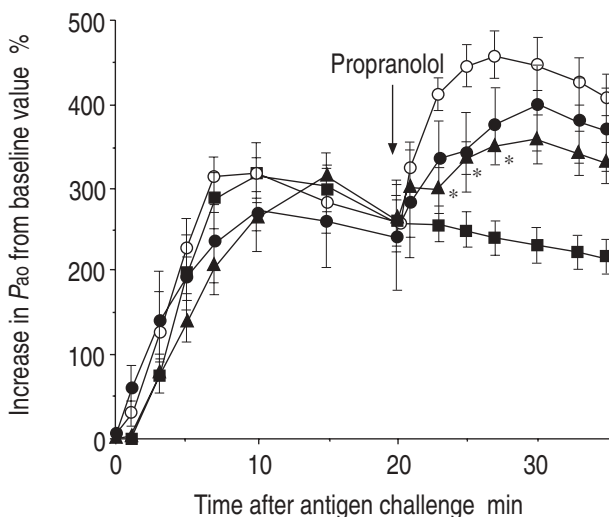


Fig. 3. — Time course of percentage increase in pressure at the airway opening (P_{ao}) from the value before ovalbumin challenge in passively sensitized guinea-pigs treated *i.v.* with vehicle (saline) ($n=8$), 1 $\mu\text{g}\cdot\text{kg}^{-1}$ E6123 ($n=8$), or 10 $\mu\text{g}\cdot\text{kg}^{-1}$ E6123 ($n=8$) 5 min before propranolol inhalation (15 min after the ovalbumin challenge). Sensitized guinea-pigs were challenged with ovalbumin, given saline *i.v.* 15 min later, and administered aerosolized saline 20 min after the ovalbumin challenge as a negative control ($n=8$). Vertical bars represent SEM. *: $p<0.05$; **: $p<0.01$, compared with the vehicle treatment group. —○—: vehicle ($n=8$); —●—: E6123 1 $\mu\text{g}\cdot\text{kg}^{-1}$ ($n=8$); —▲—: E6123 10 $\mu\text{g}\cdot\text{kg}^{-1}$ ($n=8$); —■—: control ($n=8$).

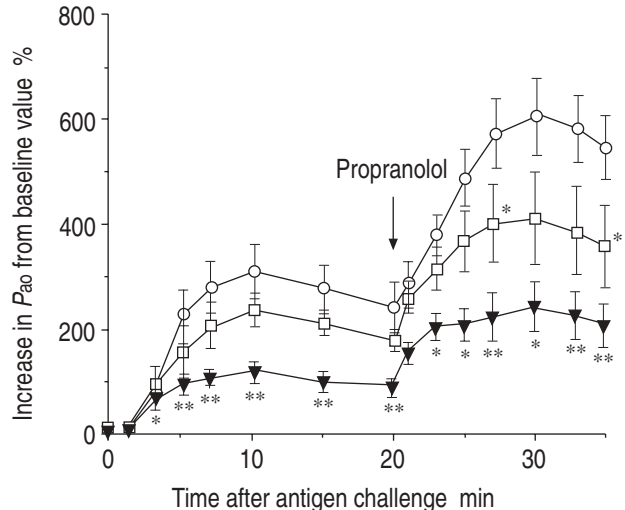


Fig. 4. — Time course of percentage increase in pressure at the airway opening (P_{ao}) in passively sensitized guinea-pigs pretreated *i.v.* with vehicle (saline) ($n=5$), 1 $\text{mg}\cdot\text{kg}^{-1}$ Y-24180 ($n=5$), or 10 $\text{mg}\cdot\text{kg}^{-1}$ Y-24180 ($n=5$), 10 min before ovalbumin challenge. Vertical bars represent SEM. *: $p<0.05$; **: $p<0.01$, compared with the vehicle treatment group. —○—: vehicle ($n=5$); —□—: Y-24180 1 $\text{mg}\cdot\text{kg}^{-1}$ ($n=5$); —▼—: Y-24180 10 $\text{mg}\cdot\text{kg}^{-1}$ ($n=5$).

Study 2: Effect of Y-24180 on propranolol-induced bronchoconstriction

Y-24180 treatment before antigen challenge. P_{ao} values before OA provocation were 10.2 ± 0.2 , 9.9 ± 0.2 and 9.9 ± 0.5 cmH_2O with pretreatment with 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 and vehicle (saline), respectively. There were no significant differences among them. The time courses of percentage increase in P_{ao} from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in figure 4. The curves both for antigen-induced bronchoconstriction (0 to 20 min after OA challenge) and propranolol-induced bronchoconstriction (20–35 min after OA challenge) were significantly inhibited by Y-24180 in a dose-dependent manner ($p<0.01$ between the three groups by two-factor repeated ANOVA). The peak values from continuous measurement after OA challenge were 323 ± 42 , 252 ± 25 and $121\pm 15\%$ with vehicle and 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180, respectively, and the value was significantly ($p<0.01$) lower with 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 than with vehicle. The maximum percentage increase in P_{ao} from continuous measurement after propranolol inhalation from the pre-OA challenge value were 611 ± 62 , 424 ± 71 and $253\pm 38\%$ with vehicle and 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180, respectively. The value with 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 was significantly ($p<0.01$) lower than that with vehicle. As shown in figure 4, 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 significantly inhibited the increases in P_{ao} both after OA challenge and after propranolol inhalation.

Y-24180 treatment after antigen challenge. P_{ao} values before OA provocation were 9.9 ± 0.3 , 10.0 ± 0.2 and 10.0 ± 0.4 cmH_2O in animals pretreated with 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 and vehicle, respectively. There were no significant differences among them. The time courses of percentage increase in P_{ao} from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in figure 5. The peak values from

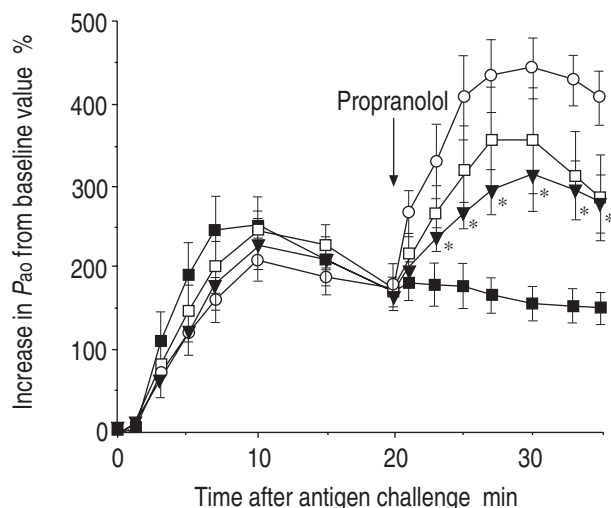


Fig. 5. — Time course of percentage increase in pressure at the airway opening (P_{ao}) from the value before ovalbumin challenge in passively sensitized guinea-pigs treated intravenously with vehicle (saline) ($n=8$), $1 \text{ mg}\cdot\text{kg}^{-1}$ Y-24180 ($n=8$), or $10 \text{ mg}\cdot\text{kg}^{-1}$ Y-24180 ($n=8$) 5 min before propranolol inhalation (15 min after the ovalbumin challenge). Sensitized guinea-pigs were challenged with ovalbumin, given saline intravenously 15 min later, and administered aerosolized saline 20 min after the ovalbumin challenge as a negative control ($n=8$). Vertical bars represent SEM. *: $p < 0.05$ compared with the vehicle treatment group. —○—: vehicle ($n=8$); —□—: Y-24180 $1 \text{ mg}\cdot\text{kg}^{-1}$ ($n=8$); —▼—: Y-24180 $10 \text{ mg}\cdot\text{kg}^{-1}$ ($n=8$); —■—: control ($n=8$).

continuous measurement after OA challenge were 210 ± 22 , 249 ± 26 and $239 \pm 21\%$ with vehicle and 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180, respectively, and there were no significant differences between them. Percentage increase in P_{ao} immediately before propranolol inhalation (20 min after the OA challenge) were 166 ± 18 , 165 ± 11 and $159 \pm 13\%$ with vehicle and 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180, respectively. These values were not significantly different. The time courses of percentage increases in P_{ao} after the inhalation of propranolol were significantly ($p < 0.05$) different between vehicle and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 groups. The maximum percentage increase in P_{ao} from continuous measurement after propranolol inhalation from the pre-OA challenge value were 464 ± 35 , 366 ± 66 and $325 \pm 40\%$ with vehicle and 1 and 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180, respectively. The value with 10 $\text{mg}\cdot\text{kg}^{-1}$ of Y-24180 was significantly ($p < 0.05$) lower than that with vehicle.

Discussion

The present study showed that E6123 and Y-24180 given intravenously 15 min after antigen challenge both significantly and dose-dependently inhibited the bronchoconstriction induced by aerosolized propranolol administered 20 min after the antigen inhalation. Pretreatment with E6123 or with Y-24180 before the antigen challenge resulted in a decrease in the airway response produced by propranolol inhalation as well as by antigen provocation.

Two selective PAF antagonists, E6123 and Y-24180, were used to examine the role of PAF in the PIB developed after allergic reaction. Median inhibitory concentration (IC_{50}) values of E6123 on ^3H -PAF binding to human and guinea-pig platelets are 2.7 and 3.0 nM, and those on PAF-induced platelet aggregation in platelet-rich plasma

of human, guinea-pig and beagle dog are 10.1, 14.7 and 16 μM , respectively [14]. Intravenous administration of E6123 causes dose-dependent inhibition of bronchoconstriction caused by intravenous injection of PAF, with an IC_{50} value of $1 \mu\text{g}\cdot\text{kg}^{-1}$ in guinea-pigs [14]. In PAF-induced human platelet aggregation Y-24180 (IC_{50} 0.84 nM) is more potent than WEB2086 (IC_{50} 4.21 nM) and etizolam (IC_{50} 998 nM) [15]. Y-24180, WEB2086 and etizolam displace bound ^3H -PAF from the washed-platelets of rabbits, with an IC_{50} value of 3.50, 9.35 and 29.5 nM, respectively [15]. Y-24180 displaces bound ^3H -diazepam binding from the synaptosomal membranes of rat cerebral cortex with an equilibrium inhibition constant (K_i) value of 3.68 μM [15]. The affinity of Y-24180 for benzodiazepine receptors is lower than those of WEB2086 and etizolam, and is about 1,000 times lower than that for PAF receptors in platelets [15]. Intravenous administration of Y-24180 at doses of 0.3–3 $\mu\text{g}\cdot\text{kg}^{-1}$ causes dose-dependent inhibition of PAF-induced bronchoconstriction in guinea-pigs, but even at a high dose of $10 \text{ mg}\cdot\text{kg}^{-1}$ it is either inactive or weakly active against the bronchoconstriction induced by histamine, serotonin, acetylcholine, arachidonic acid, bradykinin, or LTD_4 [21]. In comparison E6123 inhibits PAF inhalation-induced bronchoconstriction in guinea-pigs with a median effective dose (ED_{50}) of $1.3 \mu\text{g}\cdot\text{kg}^{-1}$, which is lower than that of Y-24180 (ED_{50} $12 \mu\text{g}\cdot\text{kg}^{-1}$) [22]. In the present study, both E6123 and Y-24180 inhibited the PIB in a dose-dependent manner, showing that PAF plays an important role in this reaction.

CARPENTIERE *et al.* [23] reported that terfenadine, a histamine H_1 -receptor antagonist, attenuated the airway responsiveness to inhaled propranolol, suggesting that histamine may be involved in PIB in asthmatics. However, no correlation was found between the degree of bronchodilation provoked by terfenadine and the magnitude of the decrease in responsiveness to propranolol [23]. Our previous study [5] revealed that TxA_2 may also play an important role in the pathogenesis of this reaction. In addition, a recent preliminary study from our group [11] showed that a 5-lipoxygenase inhibitor, AL3264, inhibited the PIB developed after allergic reaction, leading us to examine the role of another lipid mediator, PAF, in the PIB. This study clearly showed that PAF is involved in the PIB. It adds to the list of mediators that may be involved in the PIB. However, the contribution may be relatively small because the degree of inhibition of the PIB was smaller with the PAF antagonists used in this study than with TxA_2 antagonists examined in our previous study [5]. As PAF levels in bronchoalveolar lavage fluid (BALF) or bronchial tissues were not measured after the response, we do not know the detailed mechanism of the role played by PAF in the development of PIB in our animal model. Two mechanisms may be considered: 1) PAF released by allergic reaction primes airway cells, such as eosinophils and mast cells, to release PAF and other bronchoconstrictor mediators when propranolol blocks β_2 -receptors on the cells; and 2) propranolol inhalation directly stimulates release of PAF from the airway cells, which are primed by allergic processes other than antigen-induced PAF release.

Some interactions have been demonstrated between TxA_2 and PAF in guinea-pig airway. We have shown

that PAF activates TxA_2 generation but TxA_2 does not influence PAF generation in the guinea-pig airway [24]. Pretreatment of animals with S-1452, a specific TxA_2 receptor antagonist, significantly reduced the airway response produced by PAF in a dose-dependent manner, whereas pretreatment with Y-24180 did not affect the bronchoconstriction caused by a TxA_2 mimetic, STA_2 [24]. Therefore, it is likely that PAF may lead to PIB indirectly through production of TxA_2 .

Together with our previous study on thromboxane A_2 [5] and 5-lipoxygenase [11], the present study confirms that a mediator mechanism resulting from the antigen/antibody reaction is important in the pathophysiology of propranolol-induced bronchoconstriction. However, further clinical studies are needed using specific antagonists of lipid mediators to clarify whether the mediator mechanisms are relevant to clinical asthma.

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References

- Ruffin RE, MacInyre ELM, Latimer KM, Ward HE, Crockett AJ, Alpers JH. Assessment of β -adrenoreceptor antagonist in asthmatic patients. *Br J Clin Pharmacol* 1982; 13: 325–335.
- Langer I. The bronchoconstrictor action of propranolol aerosol in asthmatic subjects. *J Physiol* 1967; 190: 41–44.
- McNeill RS. The effect of propranolol on airway resistance. *Br J Anaesth* 1967; 39: 919–925.
- McDonald AG, Ingram CG, McNeill RS. Effect of beta-adrenergic-blocking agent, propranolol, on asthmatics. *Lancet* 1964; ii: 1101–1102.
- Songur N, Fujimura M, Mizuhashi K, Saito M, Xiu Q, Matsuda T. Involvement of thromboxane A_2 in propranolol-induced bronchoconstriction in guinea-pigs. *Am J Respir Crit Care Med* 1994; 149: 1488–1493.
- Mizuguchi M, Fujimura M, Amemiya T, Nishi K, Ohka T, Matsuda T. Involvement of NK_2 receptors rather than NK_1 receptors in bronchial hyperresponsiveness induced by allergic reaction in guinea-pigs. *Br J Pharmacol* 1996; 117: 443–448.
- Ind PW, Dixon CMS, Fulle RW, Barnes PJ. Anticholinergic blockade of beta-blocker-induced bronchoconstriction. *Am Rev Respir Dis* 1989; 139: 1390–1394.
- Terpsta GK, Raajomakers JAK, Wassink GA. Propranolol induced bronchoconstriction: a nonspecific side-effect of beta-adrenergic blocking therapy. *Eur J Pharmacol* 1981; 73: 107–108.
- Fujimura M, Sakamoto S, Nishi K, Saito M, Miyake Y, Matsuda T. Inhibitory effect of inhaled procaterol on anaphylactic bronchoconstriction and thromboxane production in guinea-pigs. *Clin Exp Allergy* 1991; 21: 189–194.
- Abraham WM, Burch SG, Farmer MW, Sielczak A, Ahmed A, Cortes A. A bradykinin antagonist modifies allergen-induced mediator release and late bronchial responsiveness in sheep. *Am Rev Respir Dis* 1991; 143: 787–796.
- Songur N, Fujimura M, Mizuhashi K, Saito M, Matsuda T. Effect of AL-3264 on propranolol-induced bronchoconstriction in guinea-pigs. *J Lipid Mediators Cell Signalling* 1995; 11: 175–185.
- Barnes PJ, Chung KF, Page CP. Platelet-activating factor as a mediator of allergic disease. *J Allergy Clin Immunol* 1988; 5: 919–932.
- Meade CJ, Heuer H. PAF antagonism as an approach to the treatment of airway hyperreactivity. *Am Rev Respir Dis* 1991; 143: 79–82.
- Tsunoda H, Sakuma Y, Harada K, et al. Pharmacological activities of a novel thienodiazepine derivative as a platelet-activating factor antagonist. *Arzneim Forsch (Drug Res)* 1990; 40: 1201–1205.
- Takehara S, Mikashima H, Muramoto Y, Terasawa M, Setoguchi M, Tahara T. Pharmacological actions of Y-24180, a new specific antagonist of platelet-activating factor (PAF). II. Interactions with PAF and benzodiazepine receptors. *Prostaglandins* 1990; 40: 571–583.
- Santives T, Roska A, Hensley GT, Moore VL, Fink JV, Abromoff P. Immunologically-induced lung disease in guinea-pig. 1976; 57: 582–594.
- Konzett H, Rossler R. Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch Exp Path Pharmacol* 1940; 195: 71–74.
- Jones T, Masson P, Hamel R, et al. Biological activity of leukotriene sulfones on respiratory tissues. *Prostaglandins* 1982; 24: 279–291.
- Fujimura M. Inhibitory effect of steroids on slow-reacting substance of anaphylaxis (SRS-A)-mediated bronchoconstriction in guinea-pigs. *Jpn J Allergol* 1983; 32: 365–375.
- Minami S, Okafuji K, Saga T, et al. A new quantitative inhalation apparatus for small animals. *Jpn J Chest Dis* 1983; 21: 252–268.
- Terasawa M, Aratani H, Setoguchi M, Tahara T. Pharmacological actions of Y-24180. I. A potent and specific antagonist of platelet-activating factor. *Prostaglandins* 1990; 40: 553–569.
- Sakuma Y, Tsunoda H, Shirato M, Katayama S, Yamatsu I, Katayama K. Pharmacological effects of oral E6123, a novel PAF antagonist, on biological changes induced by PAF inhalation in guinea-pigs. *Prostaglandins* 1991; 42: 463–472.
- Carpentiere G, Castello F, Marino S. Effect of oral terfenadine on the bronchoconstrictor response to inhaled propranolol in asthmatics. *Curr Ther Res* 1991; 49: 503–507.
- Saito M, Fujimura M, Ogawa H, Matsuda T. Role of thromboxane A_2 and platelet-activating factor in allergic bronchoconstriction in guinea pig airway *in vivo*. *J Lipid Mediators Cell Signalling* 1995; 11: 1–12.